REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

Claims 1-10 and 14-84 are pending in the application.

The allowability of claim 15 is acknowledged with appreciation. Applicants note that claims 1-13 and 15 have not been rejected over prior art.

Claim 1 has been amended to be in proper method format. Claims 1-10, 14 and 15 have been amended to clarify that the "physiologically acceptable salt thereof" refers to the oligoribo- or oligodeoxyribonucleotide. Claim 15 has been amended to be in independent form by incorporating the subject matter of base claim 14. No claim amendments have been made to overcome prior art or to limit the claim scope in any way. The full doctrine of equivalents applies to each element of each claim.

Basis for new claims 16-84 can be found in pages 2-5 of the originally filed application and the originally filed claims. No new matter has been added.

The objection to claims 1-15 on page 2 of the Office Action is obviated by the amendments shown above. Accordingly, withdrawal of the objection to claims 1-15 is respectfully requested.

The rejection of claims 1-15 under 35 U.S.C. § 112, second paragraph, on page 2 of the Office Action, is obviated by the amendments shown above. Accordingly, withdrawal of the Section 112, second paragraph, rejection of claims 1-15 is respectfully requested.

The rejection of claims 1-8 and 10-13 under U.S.C. § 112, first paragraph, on page 3 of the Office Action, is obviated by the amendments shown above. Accordingly, withdrawal of the Section 112, first paragraph, rejection of claims 1-8 and 10-13 is respectfully requested.

The rejection of claims 1-8 and 10-13 under U.S.C. § 101, on page 3 of the Office Action, is obviated by the amendments shown above. Accordingly, withdrawal of the Section 101 rejection of claims 1-8 and 10-13 is respectfully requested.

The rejection of claim 9 under 35 U.S.C. § 112, first paragraph, on pages 3-8 of the Office Action, is respectfully traversed. Applicants respectfully submit that the claimed invention is fully enabled for the following reasons.

The Examiner objects to claim 9 for the reason that the antisense-mediated inhibition of "Ki-67 expression *in vivo*" is allegedly not supported by the description and that it would be highly problematic to predict the efficacy of an antisense compound *in vivo* based solely on its performance *in vitro*.

The Examiner argues that a recent article by Braasch emphasizes that major obstacles persist in the art. The Examiner cites a number of factors that allegedly contribute to the assumed unpredictable efficacy of antisense compounds *in vivo*, such as poor antisense oligonucleotide access to sites within the mRNA to be targeted, difficulties with delivery to an uptake by cells of the antisense oligos, toxicity and immunological problems caused by antisense oligos, and artifacts created by unpredictable binding of antisense compounds to systemic and cellular proteins.

Applicant's respectfully do not agree with the Examiner's assertions for the following reasons.

Antisense oligonucleotide access to sites within the mRNA to be targeted

The access to target RNA is in fact one of the most serious problems in the development of antisense oligonucleotides. However, the folding of RNA and its association with cellular proteins *in vivo* and *in vitro* is identical. An antisense oligonucleotide which inhibits the expression of a target gene *in vitro* is also capable of inhibiting the same target gene *in vivo*. Accordingly there is no poor antisense oligonucleotide access to sites within the mRNA to be targeted.

Delivery of the antisense oligos to the cells and the cellular uptake

The cellular uptake of phosphothioate-oligonucleotides *in vitro* and *in vivo* is mainly influenced by the properties of the class of the substance and not by the individual sequence. In most cells lines the *in vitro* uptake is small. For this reason transfection reagents are usually used in the experimental tests. Upon administration *in vivo* the oligonucleotides are shown to occur in different tissues and in a different distribution. It is known that tissues such as kidney and liver as

well as cancerogenous tissue accumulate oligonucleotides. It is further known, that these oligonucleotides are intracellularily available. Therefore, no difficulties with the delivery of the antisense oligos to the cells or with the cellular uptake are to be expected in the *in vivo* experiments.

Toxicity and immunological problems

Regarding toxicity, it has to be noted that this is highly appreciated for tumor cells in cancerogenous tissue. Since cancerogenous tissue accumulates oligonucleotides much more than normal tissue this will lead to the death of tumor cells in cancerogenous tissue only.

Regarding immunological problems, which may be caused by antisense oligos, it has to be noted that the immunostimulatory potential of phosphothioate-oligonucleotides may be attributed to a number of specific sequence motivs (G-quartet, CpG dinucleotides, etc.). The Examiner's assertion that such an effect is undesired in particular for medical applications is not correct. In contrast to the Examiner's assertion, the immunostimulating effect of the phosphothioate-oligonucleotides may even support the antitumoral effect of the activation of the target gene.

Artifacts

In order to avoid artifacts in the experiments according to the present invention, control experiments have also been performed by Applicants.

Summarizing the above, it is evident to a person skilled in the art that there is no problem to predict the efficacy of the inventive antisense compounds *in vivo* based on the antisense-mediated inhibition of Ki-67 expression *in vitro*. The article by Braasch, thus, does not address the present situation.

Supporting experimental evidence in the form of Rule 132 Declaration

Moreover, Applicants have performed a number of *in vivo* examples demonstrating the therapeutic efficacy of the Ki-67 oligonucleotide in the animal model, an example of which is disclosed in the attached herewith Rule 132 Declaration. In this experiment, two syngeneic tumor models with different genetic background, the MB-49 bladder tumor model in C57/B6 mice and the RM-11 prostate adenocarcinoma model in BALB/c mice were used in order to determine the

effects of Ki-67 antisense treatment on tumor growth *in vivo*. The oligonucleotides were delivered continuously by subcutaneous mini-osmotic pumps. As a result of this antisense treatment the tumor growth was significantly reduced in ODN-treated RM-11 tumors and MB-49 tumors. Thus, it has been clearly demonstrated that the oligonucleotides according to the present invention are also effective *in vivo*. Accordingly, and in opposite to the Examiner's assumptions, the *in vitro* data obtained in the present invention allow a clear prediction of the *in vivo* situation including the access to target RNA of the antisense oligonucleotide, the uptake by cells of the antisense oligos as well as toxicity and immunological questions.

For these many reasons, Applicants submit that claim 9 fully complies with Section 112, first paragraph. Accordingly, withdrawal of the Section 112, first paragraph, rejection is respectfully requested.

The rejection of claim 14 under 35 U.S.C. § 103 as being anticipated (Applicants believe that the Examiner intended to stated "obvious") by any of Schlüter, Maeshima, or Duchrow in view of Baracchini is respectfully traversed. There is no motivation to combine any of Schlüter, Maeshima, or Duchrow with Baracchini for the many reasons provided below. For these reasons alone, the Section 103 rejection should be withdrawn.

Even if Schlüter, Maeshima, and Duchrow were combined with Baracchini, the claimed invention would not be taught or suggest by such a theoretical combination for the following reasons.

The closest prior art is Maeshima. Maeshima relates to the use of Ki-67 antisense oligonucleotides for the inhibition of human mesangial cell growth. Maeshima further describes the modification of antisense oligonucleotides by phosphothioate for increasing the stability of the oligodeoxynucleotides. Maeshima assumes that the investigated antisense oligodeoxynucleotides display a more antiproliferative than a cytotoxic effect.

An object of the present invention is providing oligoribo or oligodeoxyribonucleotides which are capable to be used in the treatment of tumours by destroying proliferating cells. This object has been solved by present claim 14 relating to an oligoribo or oligodeoxyribonucleotide or a physiologically acceptable

salt, characterized in that it is capable of hybridizing with the mRNA which codes for the protein Ki-67 and that it contains 22-46 nucleotides. Such an oligonucleotide is neither disclosed or suggested in Maeshima.

Maeshima describes the therapy of chronic glomerulonephritis in humans. Glomerulonephritis is caused by the infiltration of lymphocytes and by increased proliferation of mesangial cells. As a method of therapy Maeshima propose the use of Ki-67 antisense molecules. This proposal is based on the inhibition of the proliferation of mesangial cells and shall result in the inhibition or the stop of the disease. An elimination of the cells in Maeshima would be contra-indicative and thus undesired.

Moreover, Maeshima exclusively detects the effect on mesangial cells. The proliferation in these cells is temporarily inhibited by the oligonucleotide. After removal of the oligonucleotide from the cell culture the cells restart with proliferation. Thus, a cytotoxic effect of the oligonucleotides on the cells is not detectable and is also excluded by a LDH measurement in the culture supernatant.

Schlüter also does not disclose or suggest the subject matter of present claim 14. Schlüter describes the inhibition of cells of the IM-9 cell line via antigen specific antisense oligonucleotides of the protein Ki-67. The maximum length of the oligonucleotides described in Schlüter is 21 bases. Schlüter further discloses the inhibition of the incorporation of thymidin into human IM-9 cells. Schlüter does not describe the cytotoxicity of the antisense oligonucleotides used therein.

Furthermore, Durchow does not disclose or suggest the subject matter of present claim 14. Durchow describes a nucleoantigen which is defined by the antibody Ki-67 and which is associated with cell proliferation. Durchow does not disclose oligonucleotides or oligodeoxynucleotides which are capable of hybridizing with the mRNA coding Ki-67.

The many deficiencies of Schlüter, Maeshima, and Duchrow are not supplied by Baracchini. Baracchini describes antisense oligonucleotides as well as the modulation of multi drug resistance associated protein (MRP) via antisense oligonucleotides. However, Baracchini does not describe oligoribo or oligodeoxyribonucleotides which are capable of hybridizing with the mRNA coding

for Ki-67.

Summarizing the above, none of the documents Maeshima, Schlüter, Durchow and Baracchini, alone or in combination, disclose or describe the subject matter of present claim 14. A person skilled in the art would also not combine any of the documents Maeshima, Schlüter, or Durchow with Baracchini, to arrive at the subject matter of present claim 14. Accordingly, it is not obvious for a person skilled in the art to develop oligoribo- or oligodeoxyribonucleotides according to present claim 14 which are cytotoxic and capable of destroying proliferating cells. The cytotoxicity of the oligonucleotides according to present claim 14 is therefore surprising for a person skilled in the art starting from Maeshima, Schlüter, or Durchow combined with Baracchini.

A person skilled in the art would also not combine any of the cited documents to arrive at the subject matter of present claim 14 since none of these documents shares the same problem underlying the present invention. Therefore, the subject matter of present claim 14 involves an inventive step over the documents Maeshima, Schlüter, Durchow, and Baracchini alone or in combination.

In view of these many reasons, withdrawal of the Section 103 rejection is respectfully requested.

In view of the all of the rejections and objections of record having been addressed, Applicants submit that the claimed invention is in condition for allowance and Notice to that effect is respectfully requested.

Respectfully submitted,
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